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RESEARCH PAPER

Secondary metabolites in leaves of hybrid aspen are affected by the competitive status and early thinning in dense coppices

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Abstract

• ***Key message*** **Non-selective thinning of a hybrid aspen coppice stands via corridor or cross-corridor cutting impacts residual trees differently depending on their competitive status. Suppressed residual trees' metabolic profile indicates increased stress level, especially after cross-corridor thinning.**

• ***Context*** Early thinning with regular corridor harvests is proposed for the management of post-harvest re-sprouted hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) coppice stands. The selection of remaining trees is not size-based and their physiological acclimation to the post-thinning conditions is unknown.

• ***Aims*** To analyse differences in secondary metabolite profile between thinning treatments and trees competitive status.

• ***Methods*** We used an HPLC-qTOF mass spectrometer to analyse the leaf extracts of dominant and suppressed trees from stands with different thinning intensities: un-thinned control with basal area of 15.4 ± 1.52 m^2 ha^{-1}, corridor thinning with basal area of 8.5 ± 0.46 m^2 ha^{-1} and cross-corridor thinning with basal area of 3.9 ± 0.34 m^2 ha^{-1}.

• ***Results*** Competitive status and thinning treatment both had significant effects on the contents of compounds. Higher exposure to irradiance increased the contents of flavonoids and hydroxycinnamates. Corridor thinning treatments doubled the foliar contents of secondary metabolites and lowered macronutrient contents in competitively suppressed residual trees. Dominant residual trees were not affected in this respect.

• ***Conclusion*** Forest management practice and competitive status can significantly modify the metabolite profile in tree leaves. After corridor thinning of a young aspen coppice stand, the small-sized residual trees may initially respond with increased allocation to leaf chemical defence rather than to productivity.

Keywords Growth-defense trade-off · Intraspecific competition · *Populus* · Forest management · HPLC

1 Introduction

Several theoretical frameworks have been proposed to explain the trade-off between the allocation of photosynthates to growth and defence, which is reflected also by the contents of secondary metabolites (Stamp 2003; Matyssek et al. 2005; Neilson et al. 2013). In an environment rich in resources (e.g., light), the accumulated carbon would primarily be allocated to plant growth. In circumstances where some environmental factors (moderate drought or poor supply of nutrients, low temperature) limit plant growth more than photosynthesis, the assimilated carbon investment to the production of secondary metabolites increases (Herms and Mattson 1992). In addition to abiotic factors, the resource supply of individual plants is also determined by biotic factors, including competition with neighbouring plants, which can therefore play a crucial role in growth-defence trade-off (Matyssek et al. 2005). However, the physiological mechanisms underlying tree responses to competition are still poorly known (Pommerening and Sánchez Meador 2018).

Thinning is one of the major silvicultural practices that allow to reduce competition and to reallocate resources and growing space among the remaining trees (Long et al. 2004; Forrester 2019); early-stage thinning is especially

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recommended for fast-growing northern hardwood species (Rytter 2013).

One of the fastest-growing trees in Northern Europe is hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) (Tullus et al. 2012). Short-rotation hybrid aspen stands are established as plantations of pre-selected fast-growing clones. One possible management regime proposed for second-generation hybrid aspen stands (which re-generate vegetatively from root and stump sprouts) includes the combined production of assortments, where energy wood is obtained from early corridor harvests (at the age of 2–4 years), and the remaining trees are grown for a longer rotation period for the production of pulpwood and logs (Rytter 2006; Mc Carthy and Rytter 2015). The plantations are usually sparse, with about 1000 stems ha^{-1} (Tullus et al. 2012), but the post-harvest regenerating coppice stands can be dense with over 100,000 stems ha^{-1} (Rytter 2006; Hytönen 2018). However, we do not know how the physiology of hybrid aspen responds to intensive crowding and how much the growth-defence trade-off varies with the tree competitive status. Moreover, growth-defence trade-off has rarely been considered when evaluating the sustainability of different forest management practices. However, it is increasingly acknowledged to improve pathogen resistance in tree breeding (Desprez-Loustau et al. 2016).

Many classes of secondary compounds have been determined in various *Populus* spp. (Chen et al. 2009), including salicylates, tannins, phenolic acids and flavonol glucosides, but also the omnipresent jasmonic acid and its derivatives. Jasmonates (JA) and salicylates (SA) are both considered plant stress hormones. Although SA are toxic for insects (Hemming and Lindroth 1995; Chen et al. 2009 and citations therein), the SA pathway is more associated with the defence against pathogens, while JA are thought to regulate more the antiherbivore defence mechanism (Smith et al. 2009). Both abiotic and biotic stresses can influence the levels of JA and SA, including wounding, ozone, UV radiation and temperature (León et al. 2001; Glauser et al. 2008; Häikiö et al. 2008; Khan and Khan 2013; Jacobo-Velázquez et al. 2015; Julkunen-Tiitto et al. 2015; Nissinen et al. 2017). Flavonoids mainly act as antioxidants, and their synthesis is largely induced by UV irradiation (Lavola 1998; Kotilainen et al. 2008; Morales et al. 2010; Julkunen-Tiitto et al. 2015; Nissinen et al. 2017).

The aim of this study was to determine the secondary metabolite profile of leaves of young vegetatively regenerated hybrid aspen trees and to analyse the effect of early thinning on the secondary metabolite profile of competitively dominant and suppressed residual trees. We hypothesised that (i) concentrations of secondary metabolites are higher in competitively suppressed trees than in dominant trees and (ii) thinning of the hybrid aspen coppice stand decreases secondary metabolism in the remaining trees and diminishes the difference between dominant and suppressed tree secondary metabolite profiles.

2 Material and methods

2.1 Study site and experimental design

This study was carried out in a hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) regeneration experimental site in southern Estonia (58° 19′ 40″ N, 26° 33′ 16″ E, 71 m a.s.l.). The mean annual temperature of the sampling year (2017) was 6.3 °C, with a mean annual precipitation of 726 mm, which are, respectively, 9% lower and 6% higher compared to the mean of the previous 5 years (Estonian Weather Service 2018).

The experimental site was established in spring 2014 in a vegetatively regenerated hybrid aspen stand, which had emerged from root and stump sprouts after clear-cutting of a 14-year-old hybrid aspen plantation. The clear-felled stand had been established as a commercial plantation, where hybrid aspens belonging to 14 different clones were planted (1300 trees ha^{-1}) in a random mixture; therefore, the position of clones in an individual tree level is unknown; these clones originate from the same breeding program in Finland and the plant material improvement level and origin are similar (Tullus et al. 2007). The experimental area covers about 2 ha and was fenced against browsing by game. Prior to plantation establishment, the site was a cropland. The soil type that prevails homogeneously across the area is *Retic Umbrisol* (IUSS Working Group WRB 2015), which is a typical agricultural soil in this region.

The initial density of the hybrid aspen coppice stand was 94,000 trees ha^{-1}, and this did not vary significantly across the 2-ha study area (Hepner et al. 2020). The thinning trial of the hybrid aspen coppice stand started after the second growing season (early spring 2016). Areas with two different thinning intensities were created, keeping some areas as unmanaged control (Fig. 1). Thinning was done systematically using (i) the corridor method (C), where 2-m-wide corridors were cut, leaving 1-m-wide un-cut strips of trees, and where the initial density was reduced by about 2/3 to 23,000 trees ha^{-1} and (ii) the cross-corridor method (CC), where the 2-m-wide corridors were cut in two perpendicular directions, leaving 1 × 1-m uncut patches of trees, and where the initial density was reduced by about 8/9 to 9000 trees ha^{-1}. All treatments were randomly distributed across the experimental area in three 30 × 40-m sized replications. Each replication contained three circle plots with a size of 12.6 m^2 (radius = 2 m) in the control and 28.3 m^2 (radius = 3 m) in the corridor and cross-corridor treatments.

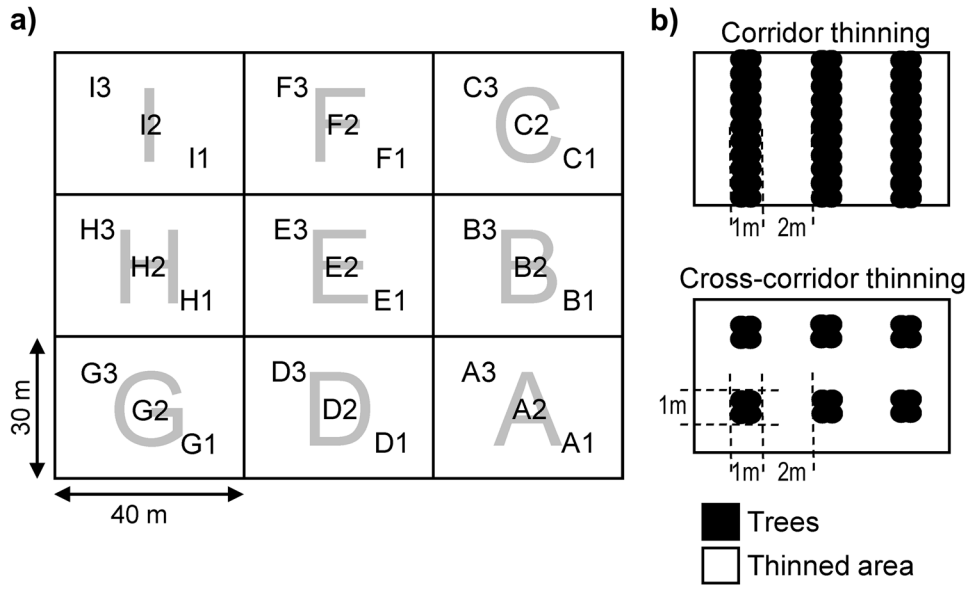

Fig. 1 **a** Design of the experimental area, where treatment replications are indicated with capital letters (A, F, H—unmanaged control; B, D, I—corridor thinning; C, E, G—cross-corridor thinning). Numbers 1, 2 and 3 indicate the circle plots in each replication. **b** Graphical illustration of the applied thinning treatments

2.2 Tree and leaf sampling

Leaf sampling was performed in mid-July 2017. Two model trees with different competitive statuses (a dominant and a suppressed tree) were chosen next to each circle plot. The model trees were selected so that the stem diameter of the dominant tree was greater than the upper diameter quartile in the given sample plot, and the diameter of the suppressed tree was less than the lower diameter quartile. Model trees were harvested, and the basic growth characteristics were measured in the field (Table 1). All leaves were separated from the model trees, and fresh weight was determined. Twenty sample leaves were randomly selected across the crown from each model tree, weighed, and dried to constant weight at 65 °C. Subsequently, the leaves were weighed to the nearest 0.01 g, and single leaf blade area was measured with the software package WinFolia (Regent Instruments Canada INC.) to estimate the mean leaf weight per area (LWA, g m^{-2}).

2.3 Foliar nutrients

The sampled leaves were ground prior to the chemical analysis. Leaf total nitrogen content ([N], %) was analysed with the Kjeldahl method, using a Kjeltec Auto 1030 Analyzer (Foss Tecator AB, Höganäs, Sweden). Phosphorus ([P], %) was determined spectrophotometrically via Kjeldahl digestion, using a FIAstar 5000 Analyzer (FOSS Tecator AB), and potassium ([K], %) was determined flame-photometrically using the Sherwood Model 425 Flame Photometer in the Laboratory of Plant Biochemistry at the Estonian University of Life Sciences.

2.4 LC-MS analysis

The extracts were chromatographically analysed using a 1290 Infinity system (Agilent Technologies, Waldbronn, Germany), coupled to an Agilent 6450 Q-TOF mass spectrometer equipped with a Jetstream ESI source.

Table 1 Growth characteristics

T	CS	H (cm)	DBH (mm)	LWA (g m^{-2})	[N] (%)	[P] (%)	[K] (%)
UT	D	569 ± 12 a	34 ± 1.2 a	73 ± 1.1 c	2.8 ± 0.06 ab	0.31 ± 0.014 ab	0.68 ± 0.07 b
C	D	553 ± 20 a	39 ± 2.5 a	84 ± 2.6 ab	2.5 ± 0.05 cd	0.28 ± 0.009 ab	0.66 ± 0.053 b
CC	D	521 ± 18 a	38 ± 1.1 a	90 ± 1.5 a	2.5 ± 0.04 bc	0.26 ± 0.008 b	0.59 ± 0.015 b
UT	S	315 ± 25 b	12 ± 1.4 b	47 ± 2.1 d	3.0 ± 0.09 a	0.34 ± 0.019 a	0.94 ± 0.039 a
C	S	304 ± 14 b	14 ± 0.9 b	66 ± 2.0 c	2.5 ± 0.06 cd	0.28 ± 0.011 ab	0.69 ± 0.030 b
CC	S	255 ± 17 b	11 ± 1.3 b	75 ± 1.5 bc	2.2 ± 0.07 d	0.25 ± 0.015 b	0.63 ± 0.017 b

(*H* height, *DBH*, stem diameter at breast height), foliar macronutrients and leaf weight per area (*LWA*) of the analysed model trees ($n = 54$), arithmetic group means ± SE, followed by letters indicating the results of Tukey's test. *T* thinning treatment (*UT* un-thinned, *C* corridor thinning, *CC* cross-corridor thinning); *CS*, tree competitive status (*D* dominant, *S* suppressed)

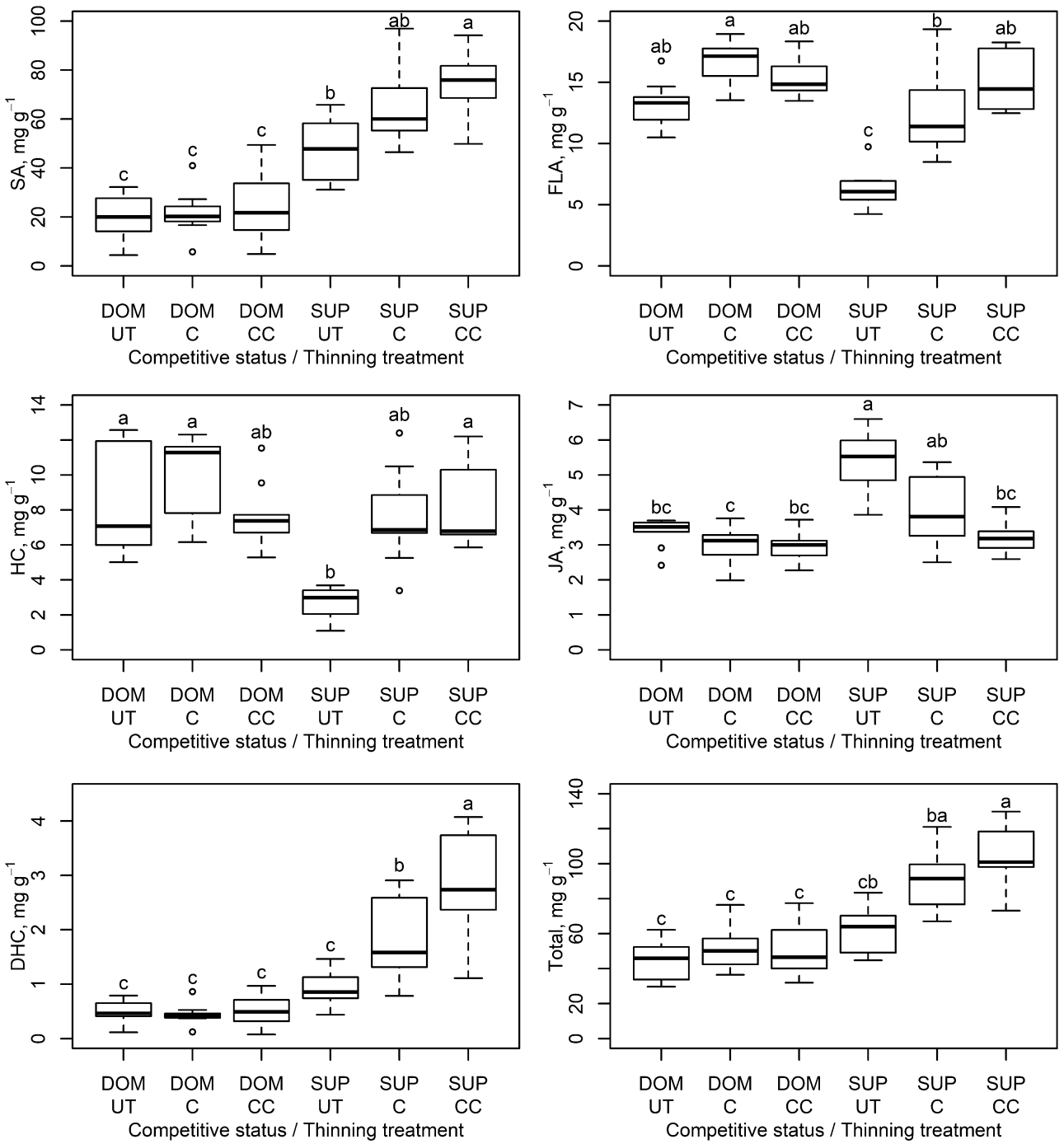

Fig. 2 Boxplots of the contents of foliar secondary metabolite classes (SA, salicylates; FLA, flavonoids; HC, hydroxycinnamates; JA, jasmonates; DHC, dihydrochalcones) by tree competitive status (DOM, dominant; SUP, suppressed) and thinning treatment (UT, un-thinned; C, corridor thinning; CC, cross-corridor thinning). Significantly different groups are distinguished with different letters, based on Tukey's test. Boxes indicate lower and upper quartile, the horizontal line marks the median and the whiskers extend to the most extreme data point, which is no more than 1.5 times the interquartile range from the box. Outliers are shown with circles

We tested 100, 80 and 50% (v/v) methanol solutions in water for extracting ground leaves, of which the 80% methanol solution gave the highest chromatographic peaks and was therefore chosen as extraction solvent. The ground plant material was extracted (1:20 w/v) for 24 h in darkness at room temperature, with occasional shaking. Subsequently, the extracts were centrifuged at 12,000*g* for 10 min with an Eppendorf MiniSpin (Eppendorf AG, Hamburg,

Germany). The obtained supernatants were subjected to a Zorbax 300SB-C18 column (2.1 × 150 mm; 5 µm; Agilent Technologies) kept at 40 °C. For the elution of the samples, a gradient of 0.1% formic acid in water (A) and acetonitrile (B) was used as follows: 0–2.0 min 1% B, 69.0 min 27% B, 72.0–79.0 min 95% B, 79.1 min 1% B, regeneration time 9 min. The eluent flow rate was set to 0.3 mL/min, and the injection volume was 3 µL. The mass spectrometer was working in negative ionisation mode in the mass-to-charge ratio (*m/z*) range of 100–1000 amu. Data acquisition and initial data processing were carried out using the MassHunter software (Agilent Technologies).

Compounds were identified by comparison of the *m/z* value, retention time, UV spectra and MS^2 fragmentation patterns with standards or by comparing data from the literature or the METLIN database (Agilent Technologies). External calibration curves of standards were used for the quantitation of compounds listed in Table 3, using MS-extracted ion chromatogram (EIC) peak areas. The generated dataset can be accessed at the following link: https://doi.org/10.5281/zenodo.4095450 (Rusalepp et al. 2020).

2.5 Chemicals

Formic acid, acetonitrile, methanol, salicin, jasmonic acid, catechin, chlorogenic acid, procyanidin B1, kaempferol-3-*O*-glucoside, rutin, myricetin and phloridzin were acquired from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

2.6 Statistical analyses

The main effects of tree competitive status (dominant or suppressed) and thinning intensity (un-thinned control, corridor thinning, cross-corridor thinning) and their interactive effect on the foliar secondary metabolites were analysed with a linear mixed effects model (LMM), where replication and sample plot were treated as random factors. The LMM analysis was performed with the function *lmer* in the package *lme4* of the R Statistics software (R Core Team 2018). The significance of the models was evaluated by comparing each full model with an intercept-only model, using the chi-square test. As several chemical constituents were analysed in each sample, Bonferroni correction for multiple testing was applied to the *p* value obtained from the chi-square test. When significant factor effects were detected, the group means were compared with Tukey's test.

Linear correlations between secondary metabolites, foliar nutrients, LWA and stand basal area (G, $m^2\ ha^{-1}$) were analysed separately for dominant and suppressed trees, based on pooled data from all treatments and plots.

Normality of model residuals was checked from residual histograms and Q-Q plots. Most of the secondary metabolite concentrations were log-transformed prior to statistical tests. A significance level of $\alpha = 0.05$ was used to reject the null hypothesis after statistical tests.

3 Results

Salicin derivatives (SA) were the most abundant class of compounds in the analysed samples (4.42–96.95 mg/g leaf dry weight), followed by flavonoids (FLA) (4.24–22.36 mg/g), hydroxycinnamates (HC) (1.09–12.57 mg/g), jasmonic acid derivatives (JA) (1.99–6.60 mg/g) and dihydrochalcones (DHC) (0.07–4.07 mg/g) (Fig. 2). The average contents of 43 compounds identified (presented in Table 5 in the Appendix) in leaves of hybrid aspens from different competitive statuses and thinning treatment classes are provided in the Table 6 in the Appendix.

The contents of the measured compounds were strongly dependent on the tree's competitive status in all compound classes (Tables 2 and 3). The total contents of different compound groups in competitively advantaged trees were not influenced by the thinning treatment (Table 2). Bivariate correlations based on data pooled by competitive status class suggest that total contents of DHC and SA were negatively correlated with

Table 2 Effects of the thinning treatment (*T*) and the tree's competitive status (*CS* dominant or suppressed) on foliar secondary metabolite groups. Significantly different treatment groups (*UT* un-thinned, *C* corridor thinning, *CC*, cross-corridor thinning) are distinguished with different letters (where 'a' is the group with the greatest mean concentration), based on Tukey's test

Class of compounds	Model	Factor			Dominant trees			Suppressed trees		
		T	CS	T×CS	UT	C	CC	UT	C	CC
Dihydrochalcones	***	*	***	***	c	c	c	c	b	a
Flavonoids	***	***	***	***	ab	a	ab	c	b	ab
Hydroxycinnamates	***	ns	***	***	a	a	ab	b	ab	a
Jasmonates	***	ns	***	***	bc	c	bc	a	ab	bc
Salicylates	***	ns	***	*	c	c	c	b	ab	a

Model—significance of the LMM model after Bonferroni correction for multiple testing ($n = 5$). *ns* not significant

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Table 3 Effects of the thinning treatment (*T*) and the tree's competitive status (*CS*, dominant or suppressed) on foliar secondary metabolites. Significantly different groups (*UT*, un-thinned; *C*, corridor thinning; *CC*, cross-corridor thinning) are distinguished with different letters (where 'a' is the group with the greatest mean concentration), based on Tukey's test. Model – significance of the LMM model after Bonferroni correction for multiple testing ($n = 43$). ns – not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

Compound	Model	Factor			Dominant trees			Suppressed trees		
		T	CS	T×CS	UT	C	CC	UT	C	CC
Dihydrochalcones										
Trihydroxy-methoxydihydrochalcone-*O*-glucoside 1	***	ns	***	ns	b	b	b	a	a	a
Trihydroxy-methoxydihydrochalcone-*O*-glucoside 2	***	ns	***	*	c	c	c	bc	ab	a
Hesperetin dihydrochalcone-*O*-glucoside 1	***	*	***	***	b	b	b	b	a	a
Hesperetin dihydrochalcone-*O*-glucoside 2	***	ns	***	***	b	b	b	b	a	a
Flavonoids										
Catechin and epicatechin	***	***	***	***	ab	a	ab	c	b	ab
(Epi)gallocatechin	***	***	***	*	a	a	a	b	a	a
Procyanidin B type	***	***	***	***	ab	a	a	c	b	a
Quercetin-*O*-pentoside 1	***	**	***	***	abc	a	ab	d	bc	c
Quercetin-*O*-pentoside 2	***	**	***	***	ab	a	a	c	b	ab
Kaempferol-3-*O*-galactoside	***	**	***	***	ab	a	ab	c	b	b
Kaempferol-3-*O*-glucoside	***	*	***	***	ab	a	ab	c	b	ab
Myricetin-*O*-pentoside	***	**	***	**	ab	a	a	d	c	bc
Kaempferol-3-*O*-glucuronide	***	*	*	***	ab	a	bc	c	a	ab
Quercetin-3-*O*-galacatoside	***	*	***	***	ab	a	ab	d	bc	c
Quercetin-3-*O*-glucoside	***	*	***	***	ab	a	ab	c	b	ab
Quercetin-3-*O*-glucuronide	***	**	***	***	ab	a	b	c	ab	ab
Myricetin-3-*O*-galactoside	ns	-	-	-	-	-	-	-	-	-
Myricetin-3-*O*-glucoside	***	***	***	**	abc	ab	a	d	cd	bc
Myricetin-3-*O*-glucuronide	***	***	***	***	ab	a	ab	c	b	ab
Kaempferol-*O*-pentosylhexoside	***	ns	**	***	ab	ab	bc	c	ab	a
Kaempferol-*O*-rhamnosylhexoside	***	ns	ns	***	a	ab	ab	b	ab	ab
Quercetin-3-*O*-arabinoglucoside	***	*	***	***	a	a	a	b	a	a
Unk flavonoid 1	***	***	***	***	ab	a	bc	c	b	bc
Unk flavonoid 2	ns	-	-	-	-	-	-	-	-	-
Hydroxycinnamates										
5-*p*-*O*-coumaroylquinic acid	***	ns	ns	***	ab	ab	bc	c	ab	a
3-*p*-*O*-coumaroylquinic acid	***	**	***	***	a	a	a	b	a	a
cis 3-*O*-caffeoylquinic acid	***	ns	***	**	a	a	ab	b	ab	ab
trans 3-*O*-caffeoylquinic acid	***	ns	***	***	a	a	a	b	a	a
5-*O*-caffeoylquinic acid	***	*	***	***	a	a	a	b	a	a
4-*O*-caffeoylquinic acid	***	ns	***	***	ab	ac	ab	cd	bd	abcd
Jasmonates										
Hydroxyl-dihydrojasmonic acids	***	ns	***	ns	a	a	a	b	b	b
Hydroxyjasmonyl sulfate	***	ns	***	*	b	ab	ab	a	ab	ab
Unidentified jasmonate	ns	-	-	-	-	-	-	-	-	-
Hydroxyjasmonic acid glucoside	***	ns	***	ns	b	b	b	a	a	a
Salicylates										
Salicin	***	ns	***	ns	b	b	b	a	a	a
Salicortin	***	ns	***	ns	b	b	b	a	a	a
Grandidentatin 1	***	***	***	**	c	bc	bc	b	a	a
Grandidentatin 2	***	*	***	***	c	bc	bc	b	a	a
Tremuloidin FA adduct	***	**	***	ns	c	d	cd	a	b	ab
Salicortin FA adduct	***	ns	***	ns	b	b	b	a	a	a
Tremulacin	***	ns	***	ns	b	b	b	a	a	a
Unknowns										
Unk1	ns	-	-	-	-	-	-	-	-	-
Unk2	***	ns	***	ns	b	b	b	a	a	a

Model—significance of the LMM model after Bonferroni correction for multiple testing ($n = 43$). *ns* not significant

$*p < 0.05$; $**p < 0.01$; $***p < 0.001$

Table 4 Linear correlations between secondary metabolite concentrations

Class of compounds	Tree's competitive status	Height	*G*	LWA	[N]	[P]	[K]
DHC	Dominant	*– 0.58*	– 0.12	– 0.11	0.13	0.25	– 0.24
FLA	Dominant	0.31	– 0.32	*0.55*	*– 0.73*	*– 0.57*	0.02
HC	Dominant	– 0.13	0.15	– 0.14	0.07	0.35	0.11
JA	Dominant	– 0.02	0.30	– 0.30	*0.52*	*0.53*	– 0.34
SA	Dominant	*– 0.57*	– 0.23	0.03	0.02	0.16	– 0.24
DHC	Suppressed	– 0.33	*– 0.63*	*0.76*	*– 0.64*	*– 0.47*	*– 0.70*
FLA	Suppressed	– 0.18	*– 0.67*	*0.86*	*– 0.82*	*– 0.66*	*– 0.78*
HC	Suppressed	– 0.01	*– 0.60*	*0.77*	*– 0.65*	*– 0.50*	*– 0.73*
JA	Suppressed	*0.43*	*0.62*	*– 0.70*	*0.64*	*0.70*	*0.69*
SA	Suppressed	– 0.26	*– 0.50*	*0.62*	*– 0.66*	*– 0.44*	*– 0.60*

(*SA* salicylates, *FLA* flavonoids, *HC* hydroxycinnamates, *JA* jasmonates, *DHC* dihydrochalcones) of dominant and suppressed trees and model tree height, stand basal area (*G*), leaf weight per area (*LWA*) and macronutrient concentrations

Significant ($p < 0.05$) correlations are in italics

the height of competitively advantaged trees (Table 4). The non-significant correlations between compounds and stand basal area (*G*) support the invariable secondary metabolism of advantaged trees, irrespective of the thinning intensity (i.e., crowding).

The thinning treatment most strongly influenced the competitively suppressed trees—DHC and FLA were significantly lower in the un-thinned control compared to both corridor thinning treatments (Table 2, Fig. 2). The levels of HC and SA differed between the cross-corridor and the un-thinned control treatment. The JA concentration in suppressed trees responded in the opposite direction, being the lowest in the cross-corridor group and the highest in the control.

Model tree height and DBH depended significantly on the tree competitive status (CS) but did not vary among the thinning treatments within a CS class (Table 1). Foliar macronutrient contents and LWA were affected by both CS as well as thinning treatment (Table 1). Stand basal area (*G*) differed significantly ($p < 0.001$) among the treatments, with average values of 15.4 ± 1.52 m^2 ha^{-1} in the un-thinned control, 8.5 ± 0.46 m^2 ha^{-1} in corridor thinning and 3.9 ± 0.34 m^2 ha^{-1} in cross-corridor thinning. Moderate to strong negative correlations were observed between *G* and contents of DHC, FLA, HC and SA in suppressed trees (Table 4). The content of JA was in moderate positive correlation with *G* and height of suppressed trees. Foliar macronutrients were significantly correlated with measured secondary compounds primarily in suppressed trees. Foliar [N], [P] and [K] levels of suppressed trees were negatively correlated with all compound classes except JA, where the respective relationships were positive. In dominant trees, foliar [N] and [P] were positively correlated with JA and negatively with FLA; these relations were slightly weaker than in suppressed trees.

4 Discussion

4.1 Exposure to irradiance affects foliar metabolite profiles of hybrid aspens on a fertile site

We present the first comprehensive study of the secondary metabolite profile of hybrid aspen leaves. Significant changes in the leaf secondary metabolite profile were detected, which were related to tree competitive status and forest management practices aimed to regulate stand density. The salicylate compound grandidentatin and its isomer, which have previously been found in some other species of the *Salicaceae* family (Pearl and Darling 1962, 1970; Si et al. 2011; Snyder et al. 2015; Jervis et al. 2015), were for the first time tentatively identified in hybrid aspen leaves.

Contrarily to our hypothesis, the expected decreased level of competition stress under improved light and nutrition conditions in the thinning treatments did not result in a lower content of foliar secondary metabolites in neither dominant nor suppressed trees. In both corridor thinning treatments (C and CC), competitively suppressed trees had higher levels of SA and DHC compared to dominant trees in all treatments, while contents of FLA, HC and JA in suppressed trees of C and CC treatments were at comparable levels with dominant trees. The only class of compounds in suppressed trees which differed significantly between the two thinning treatments was DHC, which was higher in the cross-corridor than in the corridor thinning.

Our study site lies on *Retic Umbrisol*, which is a fertile soil with high nutrient pools and good water-holding properties (Lutter et al. 2017). Therefore, dominant trees

were growing in a high-resource environment, while suppressed tree growth was obviously more limited by shading of overtopping trees than by insufficient nutrient and water supply. The observed lower levels of SA, DHC and JA in dominant trees compared to suppressed trees are in accordance with the growth-differentiation balance hypothesis, which states that in high-resource environments, the assimilated C is allocated to growth at the expense of defence mechanisms (Herms and Mattson 1992). The findings of the current study are congruent with Ruuhola and Julkunen-Tiito (2003), who showed that inducing the synthesis of SAs can also result in lower above-ground biomass accumulation for *Salix pentandra*.

Generally, dominant trees had similar levels of metabolites in all treatments. At the same time, the metabolic profiles of suppressed trees differed significantly between the treatments. At the most crowded conditions, in the un-thinned control, FLA and HC in suppressed trees were the lowest (Fig. 2). Flavonoids and hydroxycinnamates play an important role in shielding the tree leaves from UV irradiation (Burchard et al. 2000; Groenbaek et al. 2019; Valle et al. 2020), which was obviously much less intensive in the shady environment where the suppressed control group trees were growing. In suppressed trees, increases in FLA and HC contents go hand in hand with increases in the extent of thinning, supporting the association between UV radiation and FLA and HC production (Lavola 1998; Kotilainen et al. 2008; Morales et al. 2010; Nissinen et al. 2017).

DHCs belong to the class of flavonoids as well, but unlike major flavonoids (e.g. quercetin, myricetin, kaempferol), they have an open-chain structure which gives them the capacity to exhibit great antioxidant potential despite their relatively low content in plants (Nakamura et al. 2003; Dugé de Bernonville et al. 2010; Ibdah et al. 2018; Li et al. 2018). It appears that in a dense hybrid aspen coppice, competitively suppressed residual trees were put under increasing oxidative stress with the increase of thinning intensity while dominant residual trees exhibited no signs of considerable stress at all.

Four of the five classes of metabolites analysed were phenolic compounds (SA, FLA, HC and DHC) that are all derived from the phenylpropanoid pathway which requires the amino acid phenylalanine as a precursor (Dixon and Paiva 1995). JA, on the other hand, are derived from α-linolenic acid present in chloroplast membranes and are the first signalling molecules synthesised in response to various stresses (Wasternack and Strnad 2018). JA act through inhibiting plant growth by inducing the enzyme phenylalanine ammonia-lyase (PAL) that separates primary and secondary metabolism resulting in more resources allocated to the phenolic compound production (Dixon and Paiva 1995; Huang et al. 2017). JA are mostly associated with wounding and pathogen attack, but there is also evidence that oxidative stress caused by UV-B irradiation can induce the synthesis of JA (Svyatyna and Riemann 2012; Wasternack and Strnad 2018). While JA promote the synthesis of SA among other phenolic compounds by inducing PAL, SA in turn inhibits the synthesis of JA by blocking the linolenic acid pathway (Doares et al. 1995; Khan and Khan 2013; Per et al. 2018). SA blocking the synthesis of JA explains well the observed opposite correlations between phenolics and JA content in suppressed residual trees. Also, it suggests that the content of JA is inherently higher in suppressed trees than in dominant trees, since the higher content of SA suppresses the JA synthesis in the two corridor thinning treatments.

4.2 Post-thinning changes in the foliar metabolite profile reflect the altered root: shoot ratio of re-sprouted hybrid aspens

Early thinning of a young aspen coppice stand could be considered as a wounding of this root-connected clonal organism. An increase in the SA content in response to wounding has been shown in *Salix spp.* (Ruuhola et al. 2001), but it must be considered that the response to wounding is short-term, whereas the samples in the current study were collected in the middle of the second growing season after the thinning. It has been shown that the content of SA can also be enhanced by increased UV radiation (Warren et al. 2003; Nissinen et al. 2017), which would explain the raise of SA with thinning, but suppressed trees had higher levels of SA also in the control group. Controversially, Rhodes et al. (2016) found no impact of tree size on the leaf content of phenolic glucosides (mainly SA: tremulacin and salicortin) in *Populus tremuloides*, while there was a clear difference in SA content in the current study. This could be because Rhodes et al. (2016) classified trees based on stem diameter but not directly on trees competitive status within the local neighbourhood as in our study. In addition, Rhodes et al. (2016) sampled trees from older and sparser stands, where the negative impact from intraspecific competition was apparently weaker than in young and dense aspen coppices.

In a young aspen coppice forest interconnected through the root system, disturbance of one tree can trigger a physiological response in its neighbouring trees (Baret and DesRochers 2011). Moreover, defoliation of dominant trees has a greater effect on their suppressed neighbours than vice-versa (Baret and DesRochers 2011). In our study, thinning resulted in significantly lower leaf area index (reduction was 47% in the corridor and 78%

in the cross-corridor treatment) compared to un-thinned stand (Hepner et al. 2020), but it is likely that the roots of the cut trees remained alive (DesRochers and Lieffers 2001). Consequently, the maintenance cost of the root system for the remaining trees increased as they lost many root-connected neighbours, whereas the metabolic profiles revealed that post-thinning stress response was characteristic to suppressed residual trees. Knowing that suppressed trees have a smaller photosynthetic leaf area than dominant trees, thinning would more significantly affect the suppressed trees' physiology, as they have to supply the root system with photosynthates under the conditions of an even lower photosynthetic capacity than prior to thinning. Accordingly, dominant trees leaf growth efficiency (aboveground woody biomass produced per unit of leaf area) was almost twofold compared to the stand average in all treatments of our study (Hepner et al. 2020).

As we investigated a coppiced aspen stand, we lack information about the genotypes of the individual sample trees. The initial hybrid aspen plantation consisted of various genotypes with random planting when it was first established. Generally, the secondary metabolite profile of *Populus* spp. is highly dependent on the genotype (Bandau et al. 2015; Hamanishi et al. 2015; Popović et al. 2016). As suppressed and dominant trees (root-sprout originated hybrid aspens) were chosen as side-by-side pairs, it is quite likely that they shared the same parent tree root system and belonged to the same genotype. To avoid apparent spatial autocorrelation (arising from genotype and microsite), we included the sampling area as a random effect in our LMM analysis. Therefore, it can be stated with a high degree of plausibility that trees reacted differently to thinning treatment, depending on their competitive status, even despite the possible random sources of variation caused by genotype and microsite.

A higher content of secondary metabolites did not result in a significantly lower growth of trees in thinning treatments, although there was a tendency in mean tree height to decrease with increasing thinning intensity. As thinning is aimed to improve the availability of resources, we would expect to see a rise in foliar macronutrient content, but instead observed a decrease in foliar [N], [P] and [K] levels in both dominant as well as suppressed trees. It should be noted that we performed the foliar chemistry analysis in the second post-thinning year, when the trees had already had some time to acclimate to the altered environment. Generally, northern deciduous trees display fast leaf area and growth recovery responses to thinning (Rytter 2013), while the foliar secondary metabolite contents in hybrid aspen coppice stand infer that such integrated clonal tree recovery from losing neighbour trees is not as prompt as would be expected by anatomical measures. Although the FLA and HC content in suppressed trees after thinning is now comparable to that of dominant trees due to better light conditions, the almost fourfold higher SA and DHC content indicates that the trees have not yet overcome the stress that was caused by thinning.

4.3 Conclusions

For a more efficient redistribution of resources during the early management of aspen coppice forests, less intensive early thinning (resulting in a less drastic reduction in photosynthetic leaf area), with ample time for leaf area recovery, or corridor thinning followed by traditional selective thinning from below could be considered as alternatives.

The secondary metabolism of competitively dominant trees was not affected by thinning; hence, overtopping trees were growing under similar resource supply conditions irrespective of stand density. The secondary metabolism of suppressed trees showed the strongest response to thinning, which was partly related to an altered exposure to irradiance and partly to the increased maintenance cost per leaf area due to diminished support from root-connected neighbours. Several changes in suppressed trees' post-thinning metabolite profiles indicate increased stress levels. Although the increased SA content in suppressed trees enhances resistance to herbivory and pathogens it is an additional cost at the expense of tree growth. To increase biomass production, such suppressed trees should be thinned out. Further studies are, however, needed to clarify the duration of such impact within a longer time span after thinning, also including other species than hybrid aspen.

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Data availability The datasets generated and/or analyzed during the current study are available in the Zenodo repository, https://doi.org/10.5281/zenodo.4095450.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Appendix

Table 5 Retention times (t_R), mass-to-charge ratios (*m*/z) and mass differences of compounds detected in methanolic extracts of hybrid aspen leaves

	t_R (min)	Monoisotopic *m/z* $[M-H]^-$	Observed *m/z* $[M-H]^-$	Mass difference (ppm)
Dihydrochalcones				
Hesperetin dihydrochalcone-*O*-glucoside 1	41.4	465.1402	465.1392	− 2.1
Hesperetin dihydrochalcone-*O*-glucoside 2	44.4	465.1402	465.1392	− 2.1
Trihydroxy-methoxydihydrochalcone-*O*-glucoside 1	53.9	449.1453	449.1453	0.0
Trihydroxy-methoxydihydrochalcone-*O*-glucoside 2	57.1	449.1453	449.1453	0.0
Flavonoids				
(Epi)gallocatechin	5.9	305.0667	305.0665	− 0.7
Procyanidin B type	13.6	577.1351	577.1339	− 2.1
Catechin and epicatechin	14.3	289.0718	289.0718	0.0
Unk flavonoid 1	26.8		623.1254	
Myricetin-3-*O*-glucuronide	29.1	493.0624	493.0639	3.0
Myricetin-3-*O*- galactoside	29.3	479.0831	479.0845	3.1
Myricetin-3-*O*-glucoside	30.0	479.0831	479.0850	4.0
Unk flavonoid 2	31.2		623.1254	
Quercetin-3-*O*-arabinoglucoside	32.2	595.1305	595.1327	3.8
Myricetin-*O*-pentoside	33.0	449.0725	449.0730	1.2
Quercetin-3-*O*-galacatoside	34.2	463.0882	463.0895	2.8
Quercetin-3-*O*-glucuronide	34.9	477.0675	477.0696	4.4
Quercetin-3-*O*-glucoside	35.5	463.0882	463.0894	2.6
Kaempferol-*O*-rhamnosylhexoside	36.4	593.1512	593.1533	3.5
Kaempferol-*O*-pentosylhexoside	36.6	579.1355	579.1380	4.3
Quercetin-*O*-pentoside 1	37.5	433.0776	433.0786	2.3
Kaempferol-3-*O*-galactoside	38.0	447.0933	447.0950	3.8
Quercetin-*O*-pentoside 2	38.8	433.0776	433.0789	3.0
Kaempferol-3-*O*-glucuronide	39.8	461.0725	461.0730	1.1
Kaempferol-3-*O*-glucoside	40.1	447.0933	447.0942	2.0
Hydroxycinnamates				
cis 3-*O*-caffeoylquinic acid	8.8	353.0878	353.0883	1.3
trans 3-*O*-caffeoylquinic acid	9.6	353.0878	353.0867	− 3.1
5-*p*-*O*-coumaroylquinic acid	12.3	337.0929	337.0923	− 1.8
3-*p*-*O*-coumaroylquinic acid	13.2	337.0929	337.0917	− 3.6
5-*O*-caffeoylquinic acid	16.5	353.0878	353.0872	− 1.7
4-*O*-caffeoylquinic acid	18.1	353.0878	353.0871	− 2.0
Jasmonates				
Hydroxyjasmonyl sulfate	18.7	305.0700	305.0695	− 1.6
Hydroxyjasmonic acid glucoside	23.3	387.1661	387.1669	2.1
Unidentified jasmonate	43.6	373.1868	373.1879	2.9
Hydroxyl-dihydrojasmonic acid 1	63.3	227.1289	227.1297	3.5
Hydroxyl-dihydrojasmonic acid 2	66.4	227.1289	227.1293	1.8
Salicylates				
Salicin	8.5	331.1035	331.1046	3.3
Salicortin	31.2	423.1297	423.1315	4.3
Salicortin FA adduct	31.3	469.1351	469.1372	4.5
Grandidentatin 1	45.7	423.1661	423.1679	4.3
Tremuloidin FA adduct	48.4	435.1297	435.1316	4.4
Grandidentatin 2	49.4	423.1661	423.1678	4.0
Tremulacin	64.9	527.1559	527.1584	4.7
Unknown compounds				
Unk1	41.9		477.1038	
Unk2	51.7		465.1391	

Table 6 Content (mean ± SE) of identified compounds (mg g^{-1}) in leaves of dominant and suppressed hybrid aspens in different thinning treatments

Compound	Dominant trees			Suppressed trees		
	UT	C	CC	UT	C	CC
Dihydrochalcones						
Hesperetin dihydrochalcone-*O*-glucoside 1	0.12 ± 0.01	0.12 ± 0.01	0.10 ± 0.02	0.08 ± 0.01	0.23 ± 0.02	0.36 ± 0.02
Hesperetin dihydrochalcone-*O*-glucoside 2	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.18 ± 0.02	0.26 ± 0.03
Trihydroxy-methoxydihydrochalcone-*O*-glucoside 1	0.09 ± 0.02	0.07 ± 0.01	0.10 ± 0.02	0.40 ± 0.05	0.59 ± 0.13	0.88 ± 0.18
Trihydroxy-methoxydihydrochalcone-*O*-glucoside 2	0.22 ± 0.03	0.20 ± 0.03	0.26 ± 0.05	0.37 ± 0.05	0.84 ± 0.13	1.31 ± 0.13
Flavonoids						
(Epi)gallocatechin	0.23 ± 0.04	0.43 ± 0.09	0.40 ± 0.09	0.12 ± 0.09	0.19 ± 0.06	0.27 ± 0.05
Procyanidin B type	1.10 ± 0.08	1.40 ± 0.13	1.74 ± 0.13	0.38 ± 0.09	0.91 ± 0.18	1.84 ± 0.21
Catechin and epicatechin	1.83 ± 0.26	3.02 ± 0.34	3.09 ± 0.22	0.43 ± 0.15	1.99 ± 0.46	3.16 ± 0.42
Unk flavonoid 1	0.30 ± 0.01	0.43 ± 0.05	0.24 ± 0.01	0.18 ± 0.01	0.32 ± 0.03	0.22 ± 0.02
Myricetin-3-*O*-glucuronide	0.76 ± 0.06	0.98 ± 0.07	0.93 ± 0.06	0.40 ± 0.01	0.70 ± 0.10	0.80 ± 0.04
Myricetin-3-*O*- galactoside	0.61 ± 0.05	0.59 ± 0.09	0.60 ± 0.06	0.69 ± 0.08	0.76 ± 0.07	0.83 ± 0.06
Myricetin-3-*O*-glucoside	0.78 ± 0.08	0.98 ± 0.11	1.00 ± 0.08	0.36 ± 0.01	0.59 ± 0.09	0.75 ± 0.05
Unk flavonoid 2	0.54 ± 0.03	0.65 ± 0.04	0.43 ± 0.02	0.33 ± 0.05	0.61 ± 0.05	0.39 ± 0.05
Quercetin-3-*O*-arabinoglucoside	0.92 ± 0.09	0.99 ± 0.10	0.91 ± 0.12	0.42 ± 0.09	0.88 ± 0.12	1.16 ± 0.10
Myricetin-*O*-pentoside	0.75 ± 0.03	0.95 ± 0.05	0.91 ± 0.04	0.35 ± 0.01	0.55 ± 0.05	0.63 ± 0.05
Quercetin-3-*O*-galacatoside	0.80 ± 0.02	0.98 ± 0.04	0.77 ± 0.03	0.21 ± 0.03	0.56 ± 0.06	0.46 ± 0.04
Quercetin-3-*O*-glucuronide	2.02 ± 0.05	2.51 ± 0.08	1.93 ± 0.03	1.44 ± 0.08	2.26 ± 0.08	2.06 ± 0.04
Quercetin-3-*O*-glucoside	0.68 ± 0.04	0.83 ± 0.06	0.71 ± 0.05	0.21 ± 0.04	0.58 ± 0.07	0.76 ± 0.05
Kaempferol-*O*-rhamnosylhexoside	0.12 ± 0.01	0.10 ± 0.02	0.07 ± 0.01	0.07 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
Kaempferol-*O*-pentosylhexoside	0.11 ± 0.01	0.13 ± 0.01	0.09 ± 0.01	0.06 ± 0.01	0.11 ± 0.01	0.13 ± 0.01
Quercetin-*O*-pentoside 1	0.47 ± 0.02	0.62 ± 0.03	0.51 ± 0.03	0.09 ± 0.01	0.32 ± 0.04	0.34 ± 0.05
Kaempferol-3-*O*-galactoside	0.07 ± 0.004	0.09 ± 0.01	0.07 ± 0.01	0.02 ± 0.002	0.05 ± 0.01	0.05 ± 0.01
Quercetin-*O*-pentoside 2	0.18 ± 0.01	0.24 ± 0.01	0.21 ± 0.02	0.03 ± 0.004	0.12 ± 0.02	0.16 ± 0.02
Kaempferol-3-*O*-glucuronide	0.68 ± 0.03	0.95 ± 0.07	0.52 ± 0.02	0.40 ± 0.04	0.88 ± 0.06	0.71 ± 0.03
Kaempferol-3-*O*-glucoside	0.15 ± 0.01	0.19 ± 0.03	0.16 ± 0.01	0.05 ± 0.01	0.12 ± 0.02	0.18 ± 0.01
Hydroxycinnamates						
cis 3-*O*-caffeoylquinic acid	2.04 ± 0.37	2.58 ± 0.28	1.91 ± 0.20	0.60 ± 0.10	1.96 ± 0.31	2.04 ± 0.30
trans 3-*O*-caffeoylquinic acid	3.15 ± 0.30	3.48 ± 0.25	2.75 ± 0.23	1.00 ± 0.14	2.70 ± 0.31	2.74 ± 0.32
5-*p*-*O*-coumaroylquinic acid	0.56 ± 0.09	0.77 ± 0.11	0.49 ± 0.06	0.26 ± 0.03	0.86 ± 0.12	0.85 ± 0.10
3-*p*-*O*-coumaroylquinic acid	1.28 ± 0.13	1.62 ± 0.14	1.27 ± 0.09	0.36 ± 0.06	1.28 ± 0.17	1.37 ± 0.11
5-*O*-caffeoylquinic acid	0.45 ± 0.07	0.84 ± 0.13	0.56 ± 0.08	0.18 ± 0.06	0.60 ± 0.16	0.67 ± 0.12
4-*O*-caffeoylquinic acid	0.90 ± 0.18	0.59 ± 0.05	0.74 ± 0.10	0.21 ± 0.03	0.31 ± 0.05	0.63 ± 0.11
Jasmonates						
Hydroxyjasmonyl sulfate	1.10 ± 0.08	0.86 ± 0.10	0.84 ± 0.09	2.39 ± 0.30	1.32 ± 0.22	0.96 ± 0.11
Hydroxyjasmonic acid glucoside	1.84 ± 0.06	1.72 ± 0.09	1.67 ± 0.07	2.48 ± 0.10	2.27 ± 0.15	1.96 ± 0.06
Unidentified jasmonate	0.23 ± 0.01	0.22 ± 0.02	0.22 ± 0.01	0.22 ± 0.01	0.20 ± 0.02	0.20 ± 0.01
Hydroxyl-dihydrojasmonic acids	0.18 ± 0.01	0.15 ± 0.01	0.17 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
Salicylates						
Salicin	1.40 ± 0.18	0.83 ± 0.11	1.29 ± 0.19	3.74 ± 0.33	3.14 ± 0.28	3.58 ± 0.21
Salicortin	1.67 ± 0.40	2.57 ± 0.47	2.29 ± 0.63	3.67 ± 0.53	7.47 ± 0.86	9.69 ± 0.95
Salicortin FA adduct	4.76 ± 0.94	6.99 ± 1.07	6.05 ± 1.30	9.04 ± 1.06	16.36 ± 1.49	19.24 ± 1.49
Grandidentatin 1	0.22 ± 0.02	0.26 ± 0.04	0.29 ± 0.04	0.63 ± 0.26	0.98 ± 0.09	1.50 ± 0.08
Tremuloidin FA adduct	3.10 ± 0.50	0.95 ± 0.13	2.27 ± 0.51	9.71 ± 0.74	6.96 ± 0.77	7.24 ± 0.62
Grandidentatin 2	0.22 ± 0.02	0.23 ± 0.03	0.24 ± 0.04	0.39 ± 0.05	0.89 ± 0.14	1.25 ± 0.15
Tremulacin	8.34 ± 1.39	9.95 ± 1.46	12.01 ± 2.18	21.09 ± 2.10	29.46 ± 2.34	32.95 ± 1.97
Total content of secondary metabolites	45.07 ± 3.76	52.12 ± 4.02	50.88 ± 4.98	63.21 ± 4.80	91.30 ± 5.96	104.75 ± 5.83

UT un-thinned, *C* corridor thinning, *CC* cross-corridor thinning

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